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A new species and a new record of *Laccaria* (Fungi, Basidiomycota) found in a relict forest of the endangered *Fagus grandifolia* var. *mexicana*

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Abstract

Two species of *Laccaria* discovered in relicts of *Fagus grandifolia* var. *mexicana* forests in eastern Mexico are described based on the macro- and micromorphological features, and their identity supported by molecular analysis of the internal transcribed spacer (ITS) and large subunit (LSU) of the ribosomal RNA gene. The phylogeny obtained here showed that one of the Mexican species is nested in an exclusive clade which in combination with its striking morphological features, infers that it represents a new species, while the other species is placed as a member in the *Laccaria trichodermophora* clade. This is the first report in Mexico of *Laccaria* with *Fagus grandifolia* var. *mexicana* trees, with which the reported species may form ectomycorrhizal association. Descriptions are accompanied with illustrations of macro- and micromorphological characters and a discussion of related taxa are presented.

Keywords

Ectomycorrhizal fungi, ITS, Neotropical fungi, nLSU, Tricholomatales

Introduction

It has long been recognized that *Laccaria* species are important ectomycorrhizal associates of ectotrophic plants worldwide (Mueller 1992). They are known to form interactions, for example with members of the *Pinaceae*, *Dipterocarpaceae*, *Fagaceae*, *Betulaceae*, *Myrtaceae*,

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Tiliaceae and *Salicaceae* (Kropp and Mueller 1999, Wilson et al. 2013). Some species as *Laccaria laccata* and *L. bicolor* have been considered host-generalists, and inclusive, have been subject of a lot of *in vitro* experimentation worldwide. However, recent studies developed based on molecular systematics showed that under those names, complexes of species are included (Taylor et al. 2006, Jargeat et al. 2010, Vincenot et al. 2012, Sheedy et al. 2013, Popa et al. 2014). A wide ectomycorrhizal host range has also been attributed to *L. amethystina*, but in this case it has some support for its generalist abilities at the population genetics level by Roy et al. (2008), while consideration for cryptic biological species was discarded, at least among the populations sampled in France.

In the monographic work of *Laccaria* by Mueller (1992), 19 species are recognized from North America, and 40 worldwide. New or potential undescribed species from different regions, based on morphological and molecular characteristics of fructifications, or on DNA identifications of environmental samples, have been discovered recently (Wang et al. 2004, Osmundson et al. 2005, Sheedy et al. 2013, Wilson et al. 2013, 2017, Montoya et al. 2015, Luo et al. 2016, Popa et al. 2014, 2016). Nowadays, MycoBank recognizes 112 records in this group of fungi, and additionally, Wilson et al. (2017) inferred 116 phylogenetic species from 30 countries covering the known geographic range of *Laccaria*. During the advances on the systematics of the group, a small number of morphological (macro- and microscopic) features had been found taxonomically informative (McNabb 1972, Mueller 1992), which may be the cause of false interpretations, leading to conceptual misunderstandings. In fact, since early taxonomic studies on the group, the need to revise the species of *Laccaria* commonly treated under names widely cited in the literature was considered as an important task, due to the existence of different, even undescribed species, confused under apparently well-known ones, such as in the groups of *L. laccata* (Scop.) Cooke and *L. proxima* (Boud.) Pat. (Singer 1967, Mueller and Sundberg 1981, Irving et al. 1985). For example, the study by Sheedy et al. (2013) based on DNA multigene sequences, even noted that cryptic phylogenetic species were not nested as sister taxa. Thus, strict species identifications and achieving phylogenetic inferences with stronger resolution in *Laccaria*, will aid in building a robust data set, dealing with each species ectomycorrhizal host range.

In Mexico, the reports of the diversity of the genus *Laccaria* include about 17 species (Aguirre-Acosta and Pérez-Silva 1978, Bandala et al. 1988, Montoya et al. 1987, 2015, Cifuentes et al. 1990, Pérez-Silva et al. 2006, Garibay-Orijel et al. 2009). The edibility and use of some species as food has been documented (e.g. Montoya et al. 1987, Montoya-Esquivel et al. 2002, 2003, Lampman 2007, Pérez-Moreno et al. 2008) and ectomycorrhizae formed under *in vitro* culture conditions, isolated from native specimens have also been achieved (Santiago-Martínez et al. 2003, Carrasco-Hernández et al. 2010, Galindo-Flores et al. 2015). Molecular studies on most of those records are needed not only to support their identifications but for being included in phylogenetic studies. *Laccaria roseoalbescens* T. J. Baroni, Montoya and Bandala, described as new (Montoya et al. 2015) from the mesophytic forest in Veracruz, was recognized under morphological features and confirmed through phylogenetic DNA sequence analyses and recently incorporated by Luo et al. (2016) in their mo-

lecular phylogeny to confirm the distinction of the new *L. rubroalba* X. Luo, L. Ye, Mortimer & K.D. Hyde from China.

We have under research the fungal community associated to the two southernmost relicts of mesophytic forests dominated by *Fagus grandifolia* var. *mexicana* in the American Continent. This tree species is currently in danger of extinction and in the Red list of Mexican cloud forest trees, inhabiting a narrow range of nearly 145 hm^2 in Mexico (Rodríguez-Ramírez et al. 2013, Montoya et al. 2017). Taking into account its current status, we consider important to document the associated fungal species with particular focus to the ectomycorrhizal forming species. During our study, we found two species of *Laccaria* which after their morpho- and molecular analyses we concluded that with strong support can be recognized, one as *L. trichodermophora* G.M. Mueller and the other, as a distinct undescribed species close to *L. angustilamella* Zhu L., Yang & L. Wang from China. As both are part of the unknown potential mycobionts of this endangered ectotrophic tree species, we were motivated to document them.

Materials and methods

Sampling and morphological study of basidiomes

Random visits were conducted during August–September 2005 and 2007, in two stands of *Fagus grandifolia* var. *mexicana* from Veracruz, Mexico, one in Acatlán Volcano, Acatlán (19°40'43.9"N; 96°51'9.8"W, 1840 m) and the other in Mesa de la Yerba, Acajete (19°33'37.2"N; 97°01'9.8"W, 1900 m). Basidiomes of *Laccaria* growing close to *Fagus* were gathered. Macromorphological characters and color were recorded, alphanumeric color codes in descriptions refer to Kornerup and Wanscher (1967). Measurements and colors of micromorphological structures were recorded in 3% KOH. Basidiospores were studied in Melzer's reagent. Methods to determine spore ranges are those used by Montoya and Bandala (2003), with 45–50 spores measured per collection (length and width of the sporoid excluding the ornamentation) and given as a range with the symbol $\bar{X}$ representing mean values. $\bar{Q}$ represents the basidiospore length/width ratio and is given as range of mean values. Line drawings were made with a drawing tube. The examined specimens studied are deposited in XAL herbarium (acronym from B. Thiers, continuously updated; Index Herbariorum: http://sweetgum.nybg.org/ih/). The SEM images were obtained after critical point drying of pieces of lamellae previously rehydrated in ammonia, fixed in glutaraldehyde and dehydrated in an ethanol series (Bandala and Montoya 2000).

DNA extraction, PCR amplification, and sequencing

Genomic DNA of the Mexican specimen was extracted according to Montoya et al. (2014). PCR was performed to amplify the ITS (Internal Transcribed Spacer) and LSU (Large Subunit) regions of the nuclear rDNA, using primers ITS1F, ITS5/ITS4,

LR0R/LR21, LR7 (Vilgalys and Hester 1990, White et al. 1990, Gardes and Bruns 1993). PCR conditions, as well as procedures for the purification of amplified PCR products, cycle sequencing reactions and their purification were done according to Montoya et al. (2014). Once sequences were assembled and edited, they were deposited at GenBank database (Benson et al. 2017) (Table 1).

Phylogenetic methods

The phylogenetic analysis was performed with the sequences obtained in this study, as well as some retrieved from GenBank (http://www.ncbi.nlm.nih.gov/) derived from the Blast analysis (only those that best match), and complemented with related sequences used by Osmundson et al. (2005), Montoya et al. (2015) and Wilson et al. (2017) (Table 1). For this purpose, we constructed a dataset (ITS+LSU) using PhyDE v.0.9971 (Müller et al. 2010), also with MEGA 6.06 (Tamura et al. 2013) calculated the best evolutionary model and constructed the phylogenetic tree under the method of Maximum Likelihood (ML) with 500 bootstrap replications, and finally with MrBayes v 3.2.6 (Ronquist et al. 2012) constructed the phylogenetic tree (as Montoya et al. 2014) under the method of Bayesian Inference (BI). The phylogenies from ML and BI analyses were displayed using Mega 6.06 and FigTree v1.4.3 (Rambaut 2016) respectively.

Results

A total of 13 new ITS and 28S sequences for *Laccaria* were generated in this study (Table 1 and alignment in TreeBASE S21413). They were obtained from *Laccaria* samples proceeding from the two stands of *Fagus grandifolia* var. *mexicana* in the subtropical cloud forest in central Veracruz (sample AR24 comes from a conifers forest in Veracruz) (Table 1). Only bootstrap values of ≥70% and posterior probabilities (ML/PP) of ≥0.90 were considered and indicated on the tree branches. The phylogeny displayed (Fig. 1) inferred the Mexican samples clustered in two distinct clades. A group clearly related to *Laccaria trichodermophora* and another, in a separate clade, representing an undescribed species.

Taxonomy

Laccaria squarrosa **Bandala, Montoya & Ramos, sp. nov.**
MycoBank: MB823034
Figs 2–5

Holotype. MEXICO, Veracruz State, Mpio. Acatlán, Volcán de Acatlán, Aug 14 2007, DM 63 (XAL). Terrestrial under *Fagus grandifolia* var. *mexicana*.

Table 1. *Laccaria* taxa included in this study: samples, location and GenBank accession number for sequences.

Taxon	Voucher	Location	GenBank	
			ITS	28S
Cortinarius violaceus	MTS 4854 (WTU)	USA: Washington	DQ486695	DQ457662
L. alba	AWW438	China: Yunnan-Shangrila	JX504094	JX504178
L. alba	F1120750	China	JX504126	JX504206
L. alba	F1121461	China	JX504129	JX504209
L. alba	GMM6131	China: Chang Bai Shan	JX504131	JX504210
L. amethystea	FP-98556	Germany: Vorpommern	DQ499640	–
L. amethystea	TUB 011464	Germany	AF539737	–
L. amethysteo-occidentalis	AWW556	USA: California, Nevada Co.	JX504107	JX504191
L. amethysteo-occidentalis	AWW590	USA: Oregon, Benton Co.	JX504112	JX504195
L. amethystina	ALB183	China: Tibet	JX504092	JX504176
L. amethystina	F1123822	USA: Wisconsin	KU685760	KU685911
L. amethystina	GMM7041	Russia: Caucasus	KU685654	KU685797
L. amethystina	GMM7621	France: Forest comaniale de Ste. Croix	JX504150	JX504224
L. amethystina	LaAM-08-1	–	JGI Genome	JGI Genome
L. angustilamella	BAP226	China: Yunnan	JX504118	JX504201
L. angustilamella	HKAS58714	China: Yunnan, Yongping	JX504168	JX504244
L. aurantia	KUN-F 78557-Type	China: Yunnan	JQ670895	–
L. aurantia	MB-FB-101109	China: Yunnan	JQ681209	–
L. bicolor	AWW539	USA: Illinois	KM067817	KU685763
L. bicolor	AWW537	USA: Illinois, Johnson Co.	JX504105	JX504189
L. major	GMM6012	Costa Rica	KU685758	KU685909
L. major	GMM6019	Costa Rica	KU685757	KU685908
L. nobilis	F1091206	USA: Michigan	KU685636	KU685779
L. ochropurpurea	JMP0038	USA: Wisconsin	EU819479	–
L. ochropurpurea	KH_LA06_016	USA: Louisiana	KU685721	–
L. ochropurpurea	PRL3777	USA: Illinois	KU685732	JX504246
L. ochropurpurea	PRL4777	USA: Illinois	KU685733	KU685883
L. proxima	F1133825	USA: Mississippi	KU685642	KU685786
L. roseoalbescens	LM5042	Mexico: Veracruz	KJ874327	KJ874330
L. roseoalbescens	LM5099-Type	Mexico: Veracruz	KJ874328	KJ874331
L. salmonicolor	GMM7596-Type	China: Tibet	JX504143	JX504218
L. salmonicolor	GMM7602	China: Tibet	JX504145	JX504220
L. sp.	A0561	Japan: Sapporo	JX504082	–
L. sp.	A0573	Japan: Narusawa	KU685617	–
L. sp.	GMM6800	Guatemala	KU685756	KU685907
L. squarrosa[a]	DM121	Mexico: Veracruz	MF669960	MF669967
L. squarrosa[a]	DM63-Type	Mexico: Veracruz	MF669958	MF669965
L. squarrosa[a]	DM93	Mexico: Veracruz	MF669959	MF669966
L. trichodermophora	TENN42523-Type	USA: Texas	DQ149868	–
L. trichodermophora	F1111951	Costa Rica	KU685640	KU685784
L. trichodermophora	GMM7733	USA: Texas, Tyler Co.	JX504157	JX504230
L. trichodermophora	KH_LA06_013	USA: Louisiana	KM067881	KU685872

Taxon	Voucher	Location	GenBank	
			ITS	28S
L. trichodermophora	GMM7735	USA: Texas	KM067872	–
L. trichodermophora	KH-LA06-012	USA: Louisiana	KM067880	–
L. trichodermophora	GMM7734	USA: Texas	KM067871	–
L. trichodermophora	KH-LA06-007	USA: Louisiana	KM067874	–
L. trichodermophora	KH-LA06-008	USA: Louisiana	KM067875	–
L. trichodermophora	tri1125225	USA: Rocky Mountains	DQ149855	–
L. trichodermophora	KH-LA06-010	USA: Louisiana	KM067878	–
L. trichodermophora	KH-LA06-011	USA: Louisiana	KM067879	–
L. trichodermophora	KH-LA06-009	USA: Louisiana	KM067876	–
L. trichodermophora	KH-LA06-009B	USA: Louisiana	KM067877	–
L. trichodermophora	KH-LA06-004	USA: Louisiana	KM067873	–
L. trichodermophora	HC-PNNT-112	Mexico: Mexico State	KT875031	–
L. trichodermophora	GO-2009-266	Mexico: Mexico State	KC152147	–
L. trichodermophora	HC-PNNT-157	Mexico: Mexico State	KT875032	–
L. trichodermophora	GO-2009-305	Mexico: Distrito Federal	KC152149	–
L. trichodermophora	GO-2010-124	Mexico: Veracruz	KC152144	–
L. trichodermophora	EF36	Mexico	KT354980	–
L. trichodermophora	CB08167	Mexico: Mexico State	KT875029	–
L. trichodermophora	GO-2009-228	Mexico: Mexico State	KC152146	–
L. trichodermophora	GO-2010-126	Mexico: Veracruz	KC152145	–
L. trichodermophora	GO-2010-082	Mexico: Tlaxcala	KC152152	–
L. trichodermophora	GO-2009-225	Mexico: Mexico State	KC152143	–
L. trichodermophora	GO-2009-484	Mexico: Tlaxcala	KC152151	–
L. trichodermophora	HC-PNNT-192	Mexico: Mexico State	KT875033	–
L. trichodermophora	GO-2009-210	Mexico: Mexico State	KC152148	–
L. trichodermophora	HC-PNNT-132	Mexico: Mexico State	KT875030	–
L. trichodermophora	GO-2009-314	Mexico: Jalisco	KC152150	–
L. trichodermophora	HC-PNNT-099	Mexico: Mexico State	KT875034	–
L. trichodermophora	GMM7714	USA: Texas	KM067867	–
L. trichodermophora	GMM7712	USA: Texas	KM067866	–
L. trichodermophora	GMM7716	USA: Texas	KM067869	–
L. trichodermophora	HMJAU26938	–	KP128033	–
L. trichodermophora	GMM7703	USA: Texas	KM067865	–
L. trichodermophora	GMM7697	USA: Texas	KM067863	–
L. trichodermophora	GMM7698	USA: Texas	KM067864	–
L. trichodermophora[a]	Montoya 4393	Mexico: Veracruz	MF669961	MF669968
L. trichodermophora[a]	Montoya 4394	Mexico: Veracruz	MF669962	MF669969
L. trichodermophora[a]	AR24	Mexico: Veracruz	MF669964	MF669970
L. trichodermophora[a]	Bandala 4282	Mexico: Veracruz	MF669963	–
Psathyrella rhodospora	MP133 MN	–	DQ267129	AY645058

[a]samples and sequences obtained here.

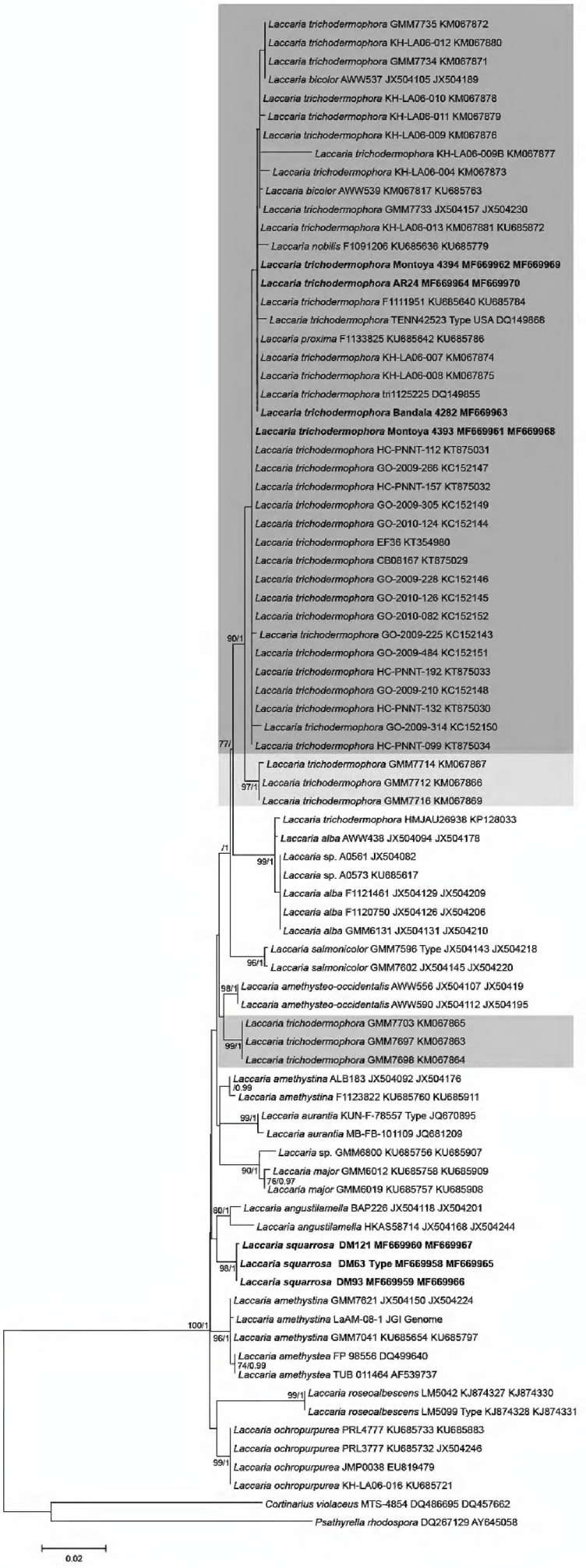
Laccaria trichodermophora GMM7735 KM067872
Laccaria trichodermophora KH-LA06-012 KM067880
Laccaria trichodermophora GMM7734 KM067871
Laccaria bicolor AWW537 JX504105 JX504189
Laccaria trichodermophora KH-LA06-010 KM067878
Laccaria trichodermophora KH-LA06-011 KM067879
Laccaria trichodermophora KH-LA06-009 KM067876
Laccaria trichodermophora KH-LA06-009B KM067877
Laccaria trichodermophora KH-LA06-004 KM067873
Laccaria bicolor AWW539 KM067817 KU685763
Laccaria trichodermophora GMM7733 JX504157 JX504230
Laccaria trichodermophora KH-LA06-013 KM067881 KU685872
Laccaria nobilis F1091206 KU685636 KU685779
Laccaria trichodermophora Montoya 4394 MF669962 MF669969
Laccaria trichodermophora AR24 MF669964 MF669970
Laccaria trichodermophora F1111951 KU685640 KU685784
Laccaria trichodermophora TENN42523 Type USA DQ149868
Laccaria proxima F1133825 KU685642 KU685786
Laccaria trichodermophora KH-LA06-007 KM067874
Laccaria trichodermophora KH-LA06-008 KM067875
Laccaria trichodermophora tri1125225 DQ149855
Laccaria trichodermophora Bandala 4282 MF669963
Laccaria trichodermophora Montoya 4393 MF669961 MF669968
Laccaria trichodermophora HC-PNNT-112 KT875031
Laccaria trichodermophora GO-2009-266 KC152147
Laccaria trichodermophora HC-PNNT-157 KT875032
Laccaria trichodermophora GO-2009-305 KC152149
Laccaria trichodermophora GO-2010-124 KC152144
Laccaria trichodermophora EF36 KT354980
Laccaria trichodermophora CB08167 KT875029
Laccaria trichodermophora GO-2009-228 KC152146
Laccaria trichodermophora GO-2010-126 KC152145
Laccaria trichodermophora GO-2010-082 KC152152
Laccaria trichodermophora GO-2009-225 KC152143
Laccaria trichodermophora GO-2009-484 KC152151
Laccaria trichodermophora HC-PNNT-192 KT875033
Laccaria trichodermophora GO-2009-210 KC152148
Laccaria trichodermophora HC-PNNT-132 KT875030
Laccaria trichodermophora GO-2009-314 KC152150
Laccaria trichodermophora HC-PNNT-099 KT875034
Laccaria trichodermophora GMM7714 KM067867
Laccaria trichodermophora GMM7712 KM067866
Laccaria trichodermophora GMM7716 KM067869
Laccaria trichodermophora HMJAU26938 KP128033
Laccaria alba AWW438 JX504094 JX504178
Laccaria sp. A0561 JX504082
Laccaria sp. A0573 KU685617
Laccaria alba F1121461 JX504129 JX504209
Laccaria alba F1120750 JX504126 JX504206
Laccaria alba GMM6131 JX504131 JX504210
Laccaria salmonicolor GMM7596 Type JX504143 JX504218
Laccaria salmonicolor GMM7602 JX504145 JX504220
Laccaria amethysteo-occidentalis AWW556 JX504107 JX50419
Laccaria amethysteo-occidentalis AWW590 JX504112 JX504195
Laccaria trichodermophora GMM7703 KM067865
Laccaria trichodermophora GMM7697 KM067863
Laccaria trichodermophora GMM7698 KM067864
Laccaria amethystina ALB183 JX504092 JX504176
Laccaria amethystina F1123822 KU685760 KU685911
Laccaria aurantia KUN-F-78557 Type JQ670895
Laccaria aurantia MB-FB-101109 JQ681209
Laccaria sp. GMM6800 KU685756 KU685907
Laccaria major GMM6012 KU685758 KU685909
Laccaria major GMM6019 KU685757 KU685908
Laccaria angustilamella BAP226 JX504118 JX504201
Laccaria angustilamella HKAS58714 JX504168 JX504244
Laccaria squarrosa DM121 MF669960 MF669967
Laccaria squarrosa DM63 Type MF669958 MF669965
Laccaria squarrosa DM93 MF669959 MF669966
Laccaria amethystina GMM7621 JX504150 JX504224
Laccaria amethystina LaAM-08-1 JGI Genome
Laccaria amethystina GMM7041 KU685654 KU685797
Laccaria amethystea FP 98556 DQ499640
Laccaria amethystea TUB 011464 AF539737
Laccaria rosecalbescens LM5042 KJ874327 KJ874330
Laccaria rosecalbescens LM5099 Type KJ874328 KJ874331
Laccaria ochropurpurea PRL4777 KU685733 KU685883
Laccaria ochropurpurea PRL3777 KU685732 JX504246
Laccaria ochropurpurea JMP0038 EU819479
Laccaria ochropurpurea KH-LA06-016 KU685721
Cortinarius violaceus MTS-4854 DQ486695 DQ457662
Psathyrella rhodospora DQ267129 AY645058
0.02

Figure 1. Phylogenetic relationships within *Laccaria* species inferred from the combined ITS and LSU sequence data by maximum likelihood method. Tree with the highest log likelihood (–4163.7219), the percentage of trees in which the associated taxa clustered together (only values ≥ 70% are considered) is shown next to the branches, followed by the posterior probabilities (only values ≥ 0.90 are indicated) obtained after Bayesian inference. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

Figure 2. *Laccaria squarrosa*, basidiomes. **a, b** habit **c, d** pileus surface details **a, c** DM 121 **b** DM 63 (holotype) **d** DM 93. Scale bars: 10 mm.

Diagnosis. *D*iffers from other species by having medium sized basidiomes, with pinkish to pale brownish-orange colors, smooth to finely squarrose surfaces, especially on the stipe, basal mycelium with whitish to pale brownish with pinkish tinges, and globose, echinulate basidiospores, 7–10 (-11.5) × 7–10.5 µm, with the echinulae 0.5–1.4 in length, 0.45–0.9 µm in width at base, subcylindrical to contorted cystidia and pileipellis arranged in a cutis with mounds of intermixed and irregularly projected hyphae.

Gene sequences ex-holotype. MF669958 (ITS), MF669965 (LSU).

Etymology. referring to the characteristic squarrose surfaces of basidiomata.

Pileus 10–82 mm diam convex to plane-convex, at times slightly depressed at center, surface squamulose to squarrose with age, pinkish (6B3–2) with pale yellowish tinges

Figure 3. *Laccaria squarrosa*, lamellae attachment and habit. **a, c** DM 121 **b** DM 63 (holotype) **d** DM 93. Scale bars: 10 mm.

towards the center or brownish-orange (5B6–5) when young; margin recurved, striate, edge thin. *Lamellae* 1–8 mm in length, adnate to subdecurrent, at times slightly undulate, subdistant or distant, pinkish to pale pinkish (6B4), 1–2 lamellulae per lamellae of different sizes. *Stipe* 50–155 × 5–9 mm, cylindrical, widened towards the base, squamulose to squarrose overall, more densely scaly towards the apex and when old, squamules brown, pinkish to ochraceous or ochraceous-orange. *Basal mycelium* pale whitish to brownish (6D6), with pinkish tinges in some areas. KOH negative overall surfaces.

Basidiospores 7–10 (-11.5) × 7–10.5 µm, $\bar{X}$=7.8–10.7 × 7.7–9.48 µm, $\bar{Q}$=1.01–1.12, globose, pale brownish, thin walled, hyaline, inamyloid, echinulate; under SEM the echinulae appear acute, 0.5–1.4 in length, 0.45–0.9 µm in width at base, shorter towards the hylar appendix area, this latter structure (also called the apiculus) consisting of a tube with rounded ending. *Basidia* 35–66 × 10–15 µm, clavate to narrowly clavate, thin walled, mostly tetrasporic, at times tri- or bisporic, sterigmata 10 µm length, some with refringent contents, clamped, hyaline. *Pleurocystidia* 20–38 × 3–6 µm, subcylindrical, contorted, sinuous, hyaline, thin walled. *Cheilocystidia* 14–40 × 2–5 µm, subcylindrical, rarely narrowly utriform, contorted, sinuous, hyaline, thin walled. *Pileipellis* a regular compact cutis, hyphae periclinally oriented, also with projected mounds of intermixed hyphae, which form the pileus scales irregularly projected; hyphae cylindrical, some widened 4–10 µm diam, some septate, hyaline, inamyloid, yellowish in

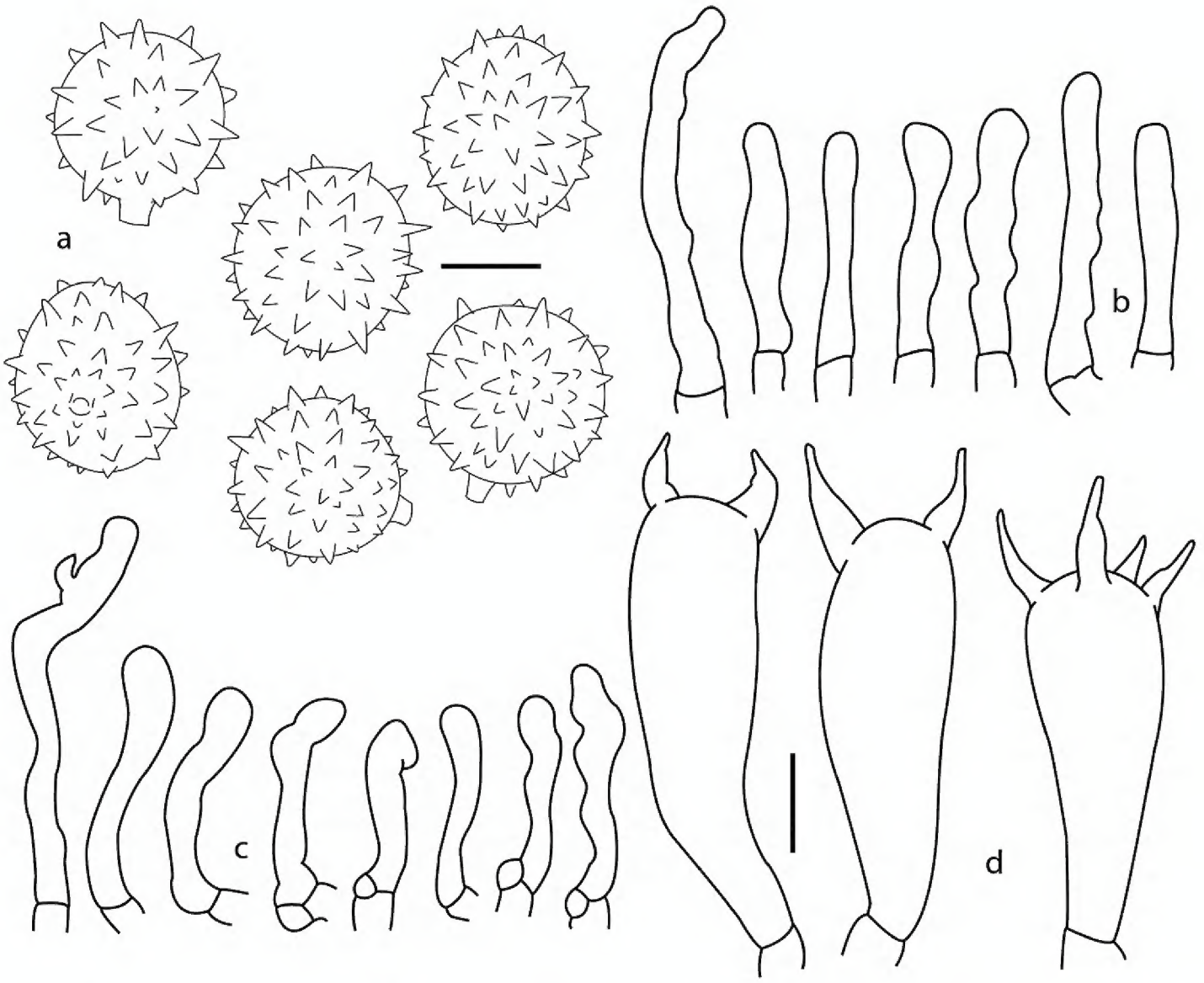

Figure 4. *Laccaria squarrosa*, **a** basidiospores **b** pleurocystidia **c** cheilocystidia **d** basidia **a, c** DM 121 **b** DM 63 (holotype) **d** DM 93. Scale bars: 5 μm (**a**); 10 μm (**b–d**).

mass and somewhat refringent in some parts, thin walled, clamped. *Context hyphae* cylindrical, faintly yellowish in mass, 4–9 (-14) μm diam, thin walled, up to 1 μm diam, hyaline, inamiloyd, septate. *Hymenophoral trama* regular, compact, composed by cylindrical hyphae, pale yellowish in mass, 3–8 μm diam, septate, hyaline and inamyloid, thin walled. *Clamps present*.

Habitat. Terrestrial, solitary, under *Fagus grandifolia* var. *mexicana.*

Additional studied material. MEXICO, Veracruz, Mpio. Acatlán, Volcán de Acatlán, Sep 18 2007, DM 121. Mpio. Acajete, Mesa de la Yerba, Aug 28 2007, DM 93 (all at XAL).

Discussion

In the phylogeny presented here that is based on sequences used in the worldwide survey of *Laccaria* by Wilson et al. (2017) and complemented with some from GenBank (Fig. 1) and sequences of *L. squarrosa*, described here, this new taxon is clearly shown to be phylogenetically isolated from other *Laccaria* species. *Laccaria squarrosa* is dis-

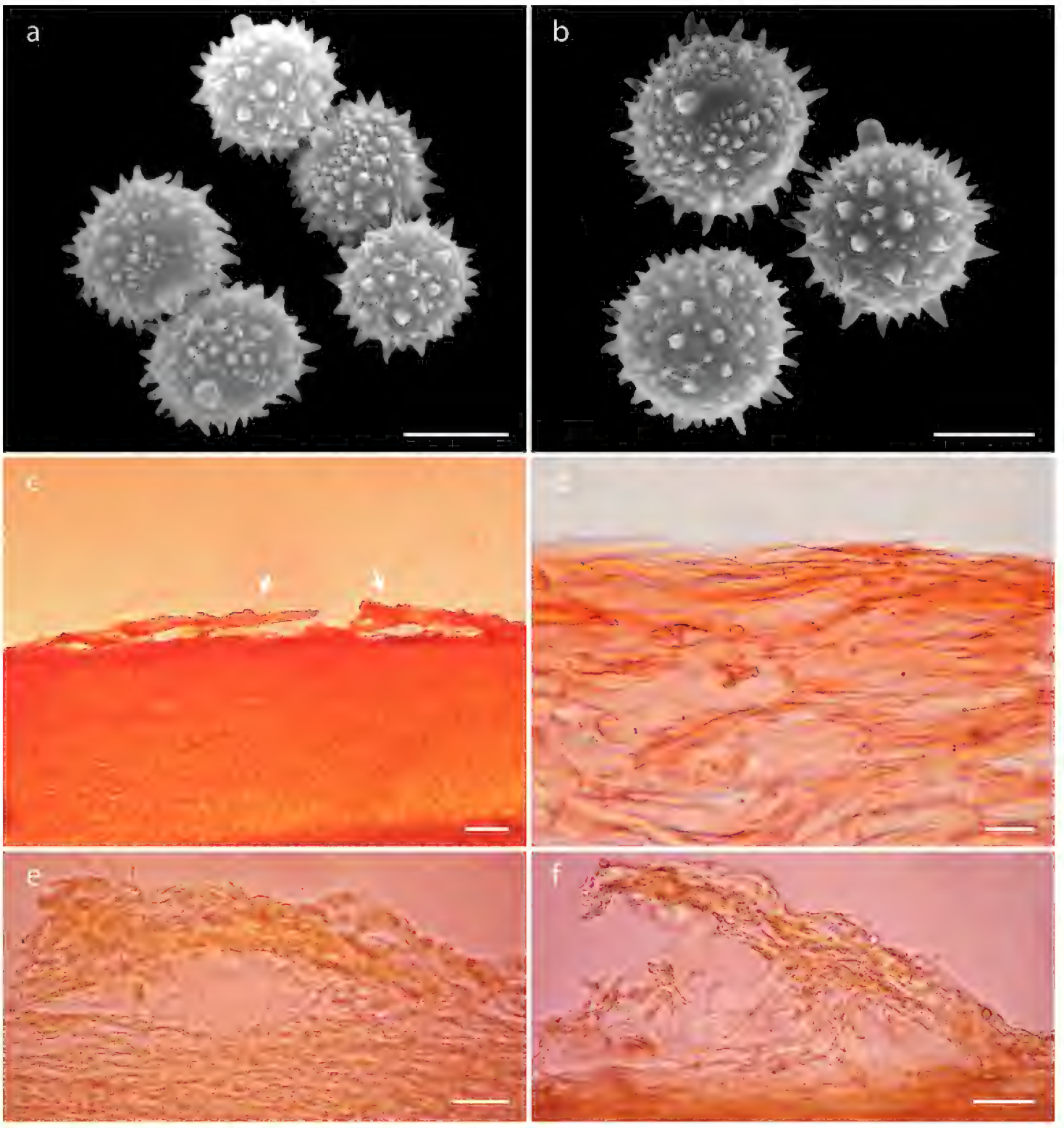

Figure 5. *Laccaria squarrosa*, **a–b** basidiospores under SEM **c–f** details of the pileipellis **c–d** cutis (arrow indicating scales) **e–f** details of the pileipellis scales **c–f** DM 63 (holotype). Scale bars: 5 µm (**a**); 2 µm (**b**); 100 µm (**c**); 20 µm (**d**); 50 µm (**e–f**).

tinct by possessing typical medium sized basidiomes with scaly surfaces, more obvious especially on the stipe and by having the basal stipe mycelium whitish to pale brownish with pinkish tinges. Microscopically it differs by globose, echinulate basidiospores, cylindrical cystidia and pileipellis arranged in a cutis with mounds of intermixed and irregularly projected hyphae. In Fig. 1, *L. squarrosa* is shown to be phylogenetically close to *L. angustilamella* Zhu L., Yang & L. Wang from China. This later species is characterized, however, by having a marasmioid to mycenoid habit, with a short basidiome size (pileus 20–30 mm diam), narrow (2 mm length) and subdistant lamellae,

non-scaly stipe, with more ellipsoid basidiospores (Q up to 1.18) and larger echinulae (2.0–) 2.5–5.0 µm long and up to 2.5 µm wide at base (Wang et al. 2004).

Color features of the basidiomes and whitish mycelia relate *Laccaria squarrosa* to metasection *Laccaria* (Mueller 1992), where it superficially resembles *L. proxima* (Boudier) Patouillard. This later species, however, can be distinguished based on the longitudinally striate stipe, with a fibrillose surface only, ellipsoid basidiospores [9–11.5 × 6.7–8 (-8.8) µm, Q = 1.25–1.35 (-1.4)], having shorter echinulae (0.5–1 µm length), pleurocystidia absence and larger cheilocystidia [19–66.5(-92) × 2–8.5(-16.5) µm] (Mueller 1992). Among the species in the genus, *Laccaria nobilis* A.H. Smith, *L. amethysteo-occidentalis* G.M. Muell., *L. trichodermophora* and *L. ochropurpurea* (Berk.) Peck also produce fibrillose to somewhat scaly pileus surfaces. *Laccaria ochropurpurea* even can have recurved scales on the stipe surface. However, all those taxa clearly differ from *Laccaria squarrosa* by basidiomes and mycelia with violaceous colors, besides other macro and microscopical features (Mueller 1992).

Laccaria trichodermophora G.M. Mueller (Figs 6–7) was previously reported from Mexico (as *L. farinacea* sensu Singer) by Montoya et al. (1987) from conifer forests of Cofre de Perote National Park areas. The collections from *Fagus grandifolia* var. *mexicana* forest here studied, were collected in the locality of Mesa de la Yerba (Veracruz), on Aug 04 2005, Montoya 4393, Montoya 4394; Aug 28 2007, Bandala 4282 (XAL). Excepting by narrower hyphae disposed in the pileipellis mounds and the basidiospores including broadly ellipsoid to ellipsoid shapes, exhibit a similar morphological variation as those described by Mueller (1984, 1992) and other collections reported before from Mexico. A summary of the main morphological features that characterize the studied materials are: *pileus* 15–55 mm diam, fibrillose to fibrillose-minutely scaly, brownish-orange (6C6–C7), light brown or pale pinkish-brown or pale brownish towards the margin (6D6, 7C5–C4), hygrophanous. *Lamellae* 2−6 mm in length, adnate to sinuate, close to subdistant, pinkish or incarnate (6A2−B3). *Stipe* 20–75 × 2–8 mm, cylindrical, attenuated towards the apex, striate, fibrillose and fistulous. hygrophanous, concolorous to pileus but more pinkish-violaceous (13CD2) towards the base. *Basal mycelium* violaceous becoming white. *Basidiospores* 6–9 (-10) × 6–8.5 (-10) µm, $\overline{X}$= 6.9–8.18 × 6.84–7.9 µm, $\overline{Q}$= 1.00–1.05, globose, hyaline, echinulate, under SEM the echinulae appear 0.8–1.13 × 0.6–0.8 µm. *Basidia* 27–65 (-80) × 7–13 µm, clavate, tetra or at times tri-sporic, hyaline, thin walled, clamped. *Cheilocystidia* 12–49 × 2–6 µm cylindrical to narrowly clavate, at times somewhat utriform, hyaline, thin walled, frequently clamped. *Pileipellis* composed of periclinally oriented hypahe 3–10 µm diam, in a more or less cutis arrangement but with frequent mounds of intermixed or erect hyphae, with terminal elements 25–125 × 10–13 µm, cylindrical to clavate other somewhat utriform 20–65 × 5–17 µm. *Clamps present*.

In the phylogeny obtained (Fig. 1), the sampled sequences of this species appear in three clades. One of them, with collections from North America, included the type specimen (DQ149868) and 21 specimens from Mexico. Our collections clustered in this later lineage interestingly with one sample from Costa Rica too. The other two clades are composed of specimens from Texas, one of them sister to the type clade, and

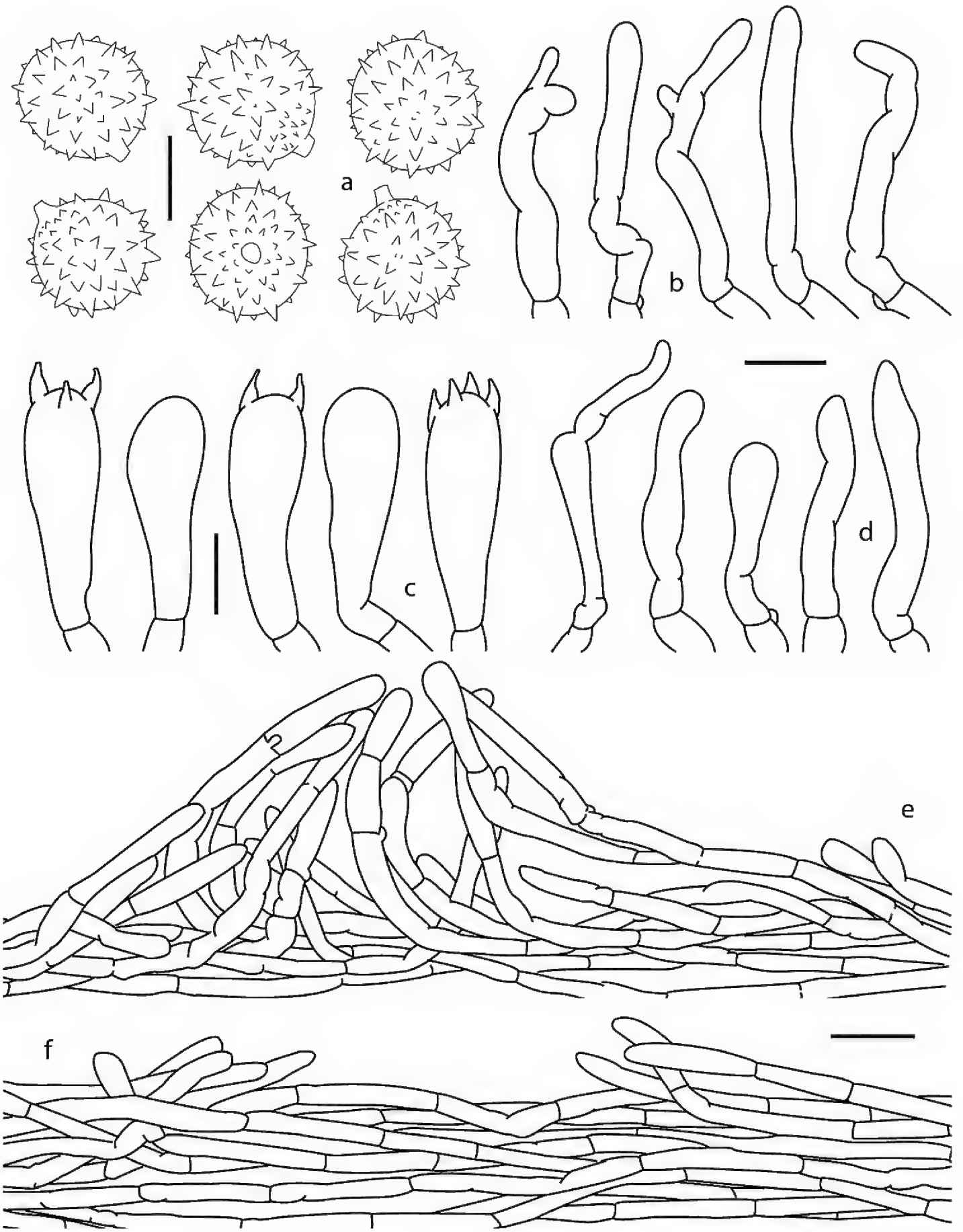

Figure 6. *Laccaria trichodermophora,* **a** basidiospores **b** pleurocystidia **c** basidia **d** pileipellis **e** cheilocystidia **a–e** Montoya 4393. Scale bars: 5 μm (**a**); 10 μm (**b–c, e**); 25 μm (**d**).

the third clearly separated, probably representing an undescribed species. A specimen (KP128033) labeled as *L. trichodermophora* in the GenBank, clustered in *L. alba* group from Asia in our analysis. This sample lacks geographic information and could well be a misidentified collection.

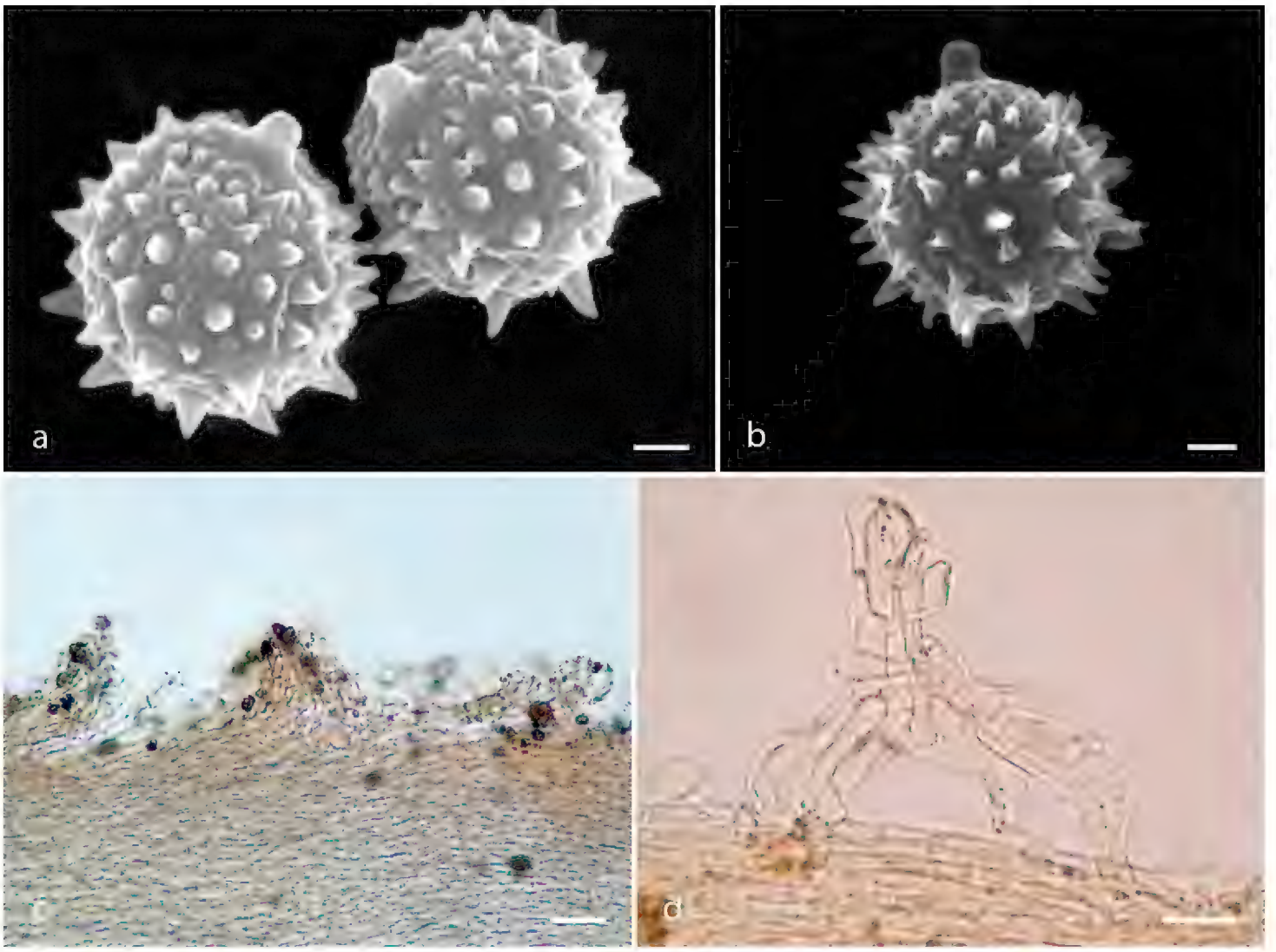

Figure 7. *Laccaria trichodermophora*, **a–b** basidiospores under SEM **c–d** pileipellis **a, c–d** Montoya 4393 **b** Bandala 4282. Scale bars: 1 μm (**a–b**); 50 μm (**c**); 25 μm (**d**).

There are no previous reports of *Laccaria trichodermophora* being associated with *Fagus grandifolia var. mexicana*. This report serves as the first documentation of this association. According to the reports of *L. trichodermophora*, it shows a wide ecological range. Mueller (1992) observed that all collections of this *Laccaria* species from the southeastern United States appeared to be associated with *Pinus*. He also collected it, in Costa Rica, beneath Neotropical species of *Quercus*. In central Mexico, in the states of Tlaxcala and Michoacán, it has been recorded associated to mixed *Pinus-Alnus* and *Pinus-Abies* forests (Montoya et al. 1987, Montoya-Esquivel et al. 2004). In the eastern part of Mexico, in Veracruz, it has been found (as *L. farinacea* sensu Singer) in monodominant *Pinus* and mixed *Pinus-Abies* forests (Montoya et al. 1987). In this later country, it is interesting to note that, basidiomes of this species, specially from conifers, are sold in local markets as edible fungi (Montoya et al. 1987, Montoya-Esquivel et al. 2004). Based on the available ecological information of the samples in the phylogenetic tree (Fig. 1), a wide host range for *L. trichodermophora* type specimen clade can be inferred. Among the potential hosts, it can be recognized as occurring with *Fagus grandifolia*, *Pinus elliottii*, *P. palustris* and *Quercus* sp. in Texas, as well as *P. patula*, other species of Pinaceae and *Quercus* spp. in both US and in Mexico, and the endangered *F. grandifolia* var. *mexicana* as confirmed here. *Abies religiosa* represents

another host also, as proved by data from two sequences (MF669964 and MF669970) (Table 1, Fig. 1) obtained here, from the sample AR24, from an *A. religiosa* forest at Cofre de Perote National Park in Veracruz, Mexico.

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